REMARKS

The Office Action of December 31, 2002 presents the examination of claims 69-85, 87-107 and 109-125. This paper cancels claims 69, 71, 79-81, 84, 86, 87, 89, 91, 93, 94, 96, 98, 102, 124 and 125, amends claims 70, 73-76, 78, 90, 92, 95, 99-101, 103, 106, 107 and 112 and presents new claims 126-135 for examination.

No new matter is introduced by any of the above amendments to the application. Nor does any of the above amendment introduce any new issue for consideration by the Examiner. Entry of the amendments is requested as the amendments place the application into condition for allowance, or in any event place the application into better condition for appeal.

Sequence Listing

The Examiner indicates that the revised Sequence Listing and Computer Readable form thereof were not received with Applicants' response of October 15, 2002. A printed Sequence Listing and Computer Readable Form thereof are attached hereto.

The revised sequences correct errors introduced in the Substitute Sequence Listing filed February 1, 1999, in which the sequences of Figure 4 were incorrectly shown. The revised Sequence Listing further incorporates the nucleic acid sequence

of GENBANK accession no. Z48542. No new matter is introduced by the revised Sequence Listing, as the corrected sequences are shown in Figure 4 and the sequence of GENBANK accession no. Z48542 is described in the text at page 3, line 27 of the specification.

The printed copy of the revised Sequence Listing is identical to the CRF copy provided on the attached diskette in the file 1380—0122P.ST25.txt, except that the CRF copy lacks formatting information.

Support for claim amendments

Most of the amendments to the claims constitute deletion of the phrase, "or functional analog thereof". In claim 112, the amendment is merely editorial. Support for the amendment of various claims to recite that the SakK or SakR genes are from a *Lactobacillus* species is provided by the specification at, e.g. page 10, lines 1-4.

SEQ ID NO: 13, with annotation, is added to the Sequence Listing. SEQ ID NO: 13 is the nucleotide sequence of the GENBANK database entry Z48542, cited at page 3, line 27 of the specification, and the annotation of SEQ ID NO: 13 (which annotation generated the sequences SEQ ID NOS:14-19 by designation of features in PATENTIN) is from that database entry. A copy of the printed database entry is attached hereto

as Exhibit A for review by the Examiner. Introduction of this sequence is not new matter as the database entry is cited as presenting the nucleotide sequence of a SakK and a SakR gene according to the invention. (The Examiner will note that the IF gene is not identified; there is no suggestion in the GENBANK entry of the existence of the IF gene or of its product. Neither is there any suggestion in the GENBANK entry of the promoter of the invention.) New claims 126-134 are supported by this disclosure.

The particular *Lactobacillus* species recited as host strains in claim 135 are supported by the specification by the Examples 1-3 at pp. 13-16.

Rejection under 35 U.S.C. § 112, first paragraph

Written Description

Claims 69-85, 87-107 and 109-125 stand rejected under 35 U.S.C. § 112, first paragraph, for alleged failure of the specification to provide adequate written description of the invention. Claims 69, 71, 78-81, 84, 86, 87, 89, 91, 93, 96, 98, 102, 124 and 125 are canceled, rendering the rejection moot as to those claims. This rejection is respectfully traversed as applied to the pending claims. Reconsideration and withdrawal thereof are requested.

The Examiner takes a position that the present application

does not adequately describe sufficient species of IF, SakK and SakR genes to support the present generic scope of the claims.

As a threshold matter, Applicants point out that the Examiner has failed to distinguish among the pending claims of various scope. For example, claims 109-111 recite none of the IF, SakK and SakR genes, but rather recite limitations of specific nucleotide sequences having a recited spacing. Claim 120 is dependent from claim 109 and adds a second feature of a peptide having a recited sequence. All of these features are explicitly shown in the Figures of the instant application and thus these claims at least should be deemed free of the instant rejection.

More substantively, the Examiner is attempting to rebut Applicants prior argument that the specification provides at least two species of each of the IF, SakK and SakR genes, "or functional analogs thereof" in support of claims so reciting a genus. The Examiner's main point is that the *PlnA*, *PlnB*, *PlnC* and *PlnD* genes exemplified are not sufficient as a second species.

The Examiner argues first that because the *PlnA* gene encodes a protein that is both an inducer and a bacteriocin and is therefore not a functional equivalent of the IF gene and thus there is inadequate support for the genus of "IF genes". The Examiner is not correct in this assertion.

It is true that practitioners in the art of bacteriocin gene expression at one time believed that the product of the *PlnA* gene was both a bacteriocin and an inducer of the bacteriocin operon. However, additional work in the field has shown that this is not correct. Applicants attach hereto a paper by E.L. Anderssen et al. (Exhibit B), which shows that in fact the product of the *L. plantarum* C11 strain is an inducer of its cognate bacteriocin operon, but does not have any significant bacteriocidal activity. The Examiner is referred specifically to the text at p. 2269, col. 2, lines 12-20, which states that,

> Originally, it was thought that these two truncated peptides compose a two-peptide bacteriocin (23). Subsequent studies revealed, however, that all three variants of *PlnA* function as a peptide hormone that induces transcription of the *pln* genes (3,6,15). The bacteriocin activity originally attributed to the PlnA peptides was presumably caused at least in part by previously unidentified bacteriocins, probably two-peptide bacteriocins (see below).

Anderssen et al. thus clarify that in fact PlnA is both a peptide inducer of the *pln* operon, in a fashion similar to the function of IF as an inducer of the *sak* operon, and also lacks

significant bacteriocin activity[1], again like IF. PlnA should be considered a functional equivalent of IF as stated in the specification.

The Examiner next argues that, because the specification does not make clear whether the *PlnC* or *PlnD* gene has the function of SakR, there is not sufficient indication of a structure-function relationship to define the genus of SakK and SakR genes. First, Applicants would point out that the Examiner's argument applies, if at all, only to the genus of SakR genes. Second, the specification makes clear that the *PlnABCD* gene cluster functions as a whole in the same manner as the IF-K-R cluster. (See, p. 7, lines 15-23.) The specification also very well describes the enzymatic activity to be exhibited by the gene products in the regulatory network. It is not beyond the skilled artisan to determine whether either or both of the *PlnC* or *PlnD* gene products should be substituted for the *SakR* gene product. In any event, to advance prosecution, Applicants have at present removed reference to "functional analogs thereof" in reference to the IF, SakK and SakR products.

As to the scope of the promoter, the Examiner has

[1]Table 1 of Andersson shows PlnA exhibits some minimal bacteriocin activity against some bacterial strains. The authors conclude at p. 2271, col. 2, lines 6-8 that PlnA is clearly a less potent antagonist than other bacteriocins and that the biological significance of its bacteriocidal activity is uncertain. Furthermore, the authors note the lack of any corresponding immunity gene, a hallmark of bacteriocin operons, in the Pln operon. (*E.g.*, *nisI* in the nisin operon, see, Fig. 2 of Kleerebezem et al. (1999), attached as Exhibit C.

previously conceded that the application discloses the common structural feature of the promoter. (*See*, the previous Office Action, p. 3, last paragraph.) The Examiner now takes a position that the specification only describes the structure of about 20% of the promoter and that the specification does not specify whether the structure that is described is correlated with a function of binding a repressor or an activator. (*See*, the paragraph bridging p. 4-5 of the Office Action.)

Applicants disagree. First, the structural description of the promoter provided in the claims is entirely sufficient to specifically identify a promoter of the invention. As evidence of this, Applicants provide attached Exhibit D ("Resultats de PATTERN6.doc"), results of a search of the GENBANK database of bacterial genomic sequences conducted under the direction of the Inventor. The search pattern reflects the language of the instant claims describing the claimed promoter. Apparent discrepancies in the "gap" parameter compared to the spacings recited in the claims are due to the way the search program must count gap bases and are not relevant. The search program counts the number of bases between the completely defined motifs, e.g. "motif 1" and "motif 2" (12-18, "motif 1" and "motif 2" are the sequences boxed in Figure 4 and encompass the SEQ ID Nos: specifically recited in the claims) whereas the claims recite spacing reflecting the variability in length of the repeated

sequence that is recited. The Examiner should note that the search results in "hits" of only the Sakacin gene promoters from *Lactobacillus sake* and Plantaracin gene promoters from *Lactobacillus plantarum*. Thus, the structure of the promoter described in the claims is clearly biologically relevant and defines a recognizable genus.

Second, the Examiner asserts a length of the promoter element of 80 nucleotides without any evidence whatsoever to support her claim that so long a sequence is necessary to the function of the inducible element described in Figure 4 of the present application. The Examiner must accept as true statements made in the specification unless she can present objective, scientifically sound reasons or evidence to doubt them. *In re Marzocchi and Horton*, 169 USPQ 367 (CCPA 1971). The Examiner has produced no evidence or reasoning to support her doubt of Applicants' statements that the minimal promoter structure shown in Figure 4 is functional to bind a regulatory protein, which binding is regulated by the activated R gene product.

Furthermore, Applicants submit that the Examiner will not be able muster such reasoning or evidence. It is well-known in the art that protein binding sites on DNA can be as small as six nucleotides and furthermore, that the protein interaction sites on many regulatory elements are constituted by short repeated

sequences separated by one to two turns of a DNA helix, i.e. 17 to 23 nucleotides as illustrated in Figure 4. In this regard, Applicants also provide Exhibits E (P.A. Riosen et al., *Mol. Microbiol.* 37:619-628 (2000) and F (P.A. Riosen et al., *Mol. Gen.* Genomics 265:198-206 (2001)). These exhibits provide data from experiments showing protein-DNA interactions in the promoter region of the *Spp* operon (an alternate naming of the present *Sak* operon, Exhibit E) and the *Pln* operon (Exhibit F). In Figure 3 of Exhibit E, gel mobility shift assays are used to show that purified regulatory proteins SppR (=SakR), or PlnC or PlnD, bind to promoter element probes of about 60 nucleotides that encompass the repeat elements (shown as LR and RR in Figure 2) disclosed in the present specification in Figure 4. This establishes i) that the short DNA sequence encompassing the repeats is sufficient for binding of the regulatory proteins and ii) that the PlnC and PlnD proteins are functionally equivalent to the SppR (SakR) protein. In Exhibit F, Figure 3 shows the result of mutation of the consensus repeat sequence upon binding of PlnC protein. The Examiner will note that the change of the 5′ flanking (-1) G residue to A, or of the 3′ flanking (+2) G residue to A has no significant effect on protein binding. On the other hand, mutation of residue C3 within the repeat completely abolishes protein binding. Similarly, mutation of residue T6 has a strong effect. Thus, these two residues are

implicated as protein contact sites. Figure 6 of Exhibit F illustrates DNAseI footprinting experiments using the Pln operon promoter and PlnC and PlnD as the DNA binding proteins. The protected portions of the probe are two short sequences, each of which covers one of the repeat sequences illustrated in the promoter in Figure 4 of the specification.

The data in Exhibits E and F establish that the functional region of the promoter according to the instant invention is defined by the repeat sequences and their spacing as illustrated in Figure 4 of the instant specification. Thus, the Examiner's argument that the specification does not adequately describe the structure function relationship of the promoter element recited in the claims is entirely without merit.

Also, it is irrelevant that the specification does not choose between release of a repressor or binding of an activator. There is no requirement that the Applicant know the theory by which his invention works. *Micro Motion Inc. v. Exac Corp.* 16 USPQ2d 1001 (DC NCalif), citing *Diamond Rubber Co. v. Consolidated Rubber Tire Co.*, 220 U.S. 428, 435-36 (1911); *Raytheon Company v. Roper Corporation*, 220 USPQ 592 (Fed. Cir. 1983).

Applicants submit that, as the Examiner has admitted in the first instance, the specification adequately describes the structural elements of a promoter effective in the instant

invention that are correlated with the function of regulation of the promoter by the IF-K-R cascade. Accordingly, claims limited by the structure of the promoter should be found to be of scope adequately supported by the specification and free of the instant rejection. This is especially true of claims 107, which is directed particularly to the promoter as an isolated nucleic acid, 109-111, directed to a vector comprising the promoter, and 120, directed to a kit comprising the vector.

Enablement

Claims 69-85, 87-107 and 109-125 stand rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement by the specification. Claims 69, 71 77, 84, 86, 87, 89, 91, 93, 96, 98, 102, 124 and 125 are canceled, rendering this rejection moot as to those claims. This rejection is respectfully traversed as to the pending claims. Reconsideration and withdrawal thereof are requested.

The Examiner argues that the claims define, and the specification describes, only 20% of the promoter sequence that provides for inducibility of the IF-K-R gene cluster and that there is no guidance with respect to the remainder of the structure as to modification that can be performed with retention of function. The Examiner asserts that the claims encompass promoters of low homology to the sequences stated and

does not establish any predictable scheme for modifying nucleotides of the promoter with retention of function.

Applicants first note that the Examiner's beginning premise is incorrect, as has been explained above. The structure of the claimed promoter is explicit in the claims, the Examiner provides no basis for her assertion that any feature of the claimed nucleic acid is a nucleotide sequence longer than that set forth in the claims.

Furthermore, the Examiner's rejection is merely speculation. The Examiner provides no reasoning and no evidence that addition of sequences to either end of the recited structure would interfere with efficacy of the regulatory element. The Examiner provides no evidence that the particular sequence of nucleotides in the region between the repeat sequences in any way affects efficacy of the regulatory element.

Still further, it was well within the skill of the artisan as of the priority date of the instant application to make and test variant promoter sequences, e.g. by site-directed mutation or by deletion analysis using a *Lactobacillus* host to assay inducibility of the promoter using expression of a reporter gene. Such experimentation was typical in the art and expected to have to be performed in testing variant gene structures for activity. Applicants attach Exhibit G, an excerpt from a textbook published in 1992 (pp. 154-155 of "Recombinant DNA",

J.D. Watson et al., c. 1992 by Scientific American Books), fully 4 years prior to the filing date of the instant application, as evidence such assays were well-known in the art. Thus, the skilled artisan can utilize the specification, the knowledge in the art at the time the invention was made, and expected experimentation, to perform the invention throughout its full claimed scope. The specification is thus an enabling disclosure. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

Still further, Applicants do not utilize the term "homology" in the claims; two sequences are recited, having a relation of a recited range of spacing. Applicants submit that the skilled artisan is well-enabled to create a promoter element having these features.

The Examiner further argues, as to claim 109, that the skilled artisan does not know the inducer that is effective for regulating the promoter. Applicants are not certain what is intended by this argument and it is entirely without merit. The specification clearly indicates that the IF peptide of Figure 2 is an example of an effective "inducer" and that the SakR gene product of *L. sake* ultimately acts at the described promoter. Also, the PlnA gene product from *L. plantarum* C11 is described as an effective inducer and that the *PlnC* and or *PlnD* gene product will also be effective in ultimate action at the described promoter.

Applicants submit that the invention as presently claimed, using the teaching of the specification, the knowledge of the art at the time the invention was made and expected experimentation, can be practiced without undue experimentation throughout the scope of the claims. Thus, the specification is enabling of the claimed invention and the instant rejection should be withdrawn.

Rejection under 35 U.S.C § 112, second paragraph

Claims 69-85, 87-107 and 109-125 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested. In particular, the Examiner asserts that the claims recite IF, SakK, SakR, PlnA, PlnB, PlnC, etc. [genes] and it is not clear to the Examiner what molecules are encompassed by these designations. The Examiner further states that the phrase "of a lactic acid bacterium" in some claims is confusing because it may imply that genes not from a lactic acid bacterium are encompassed.

From the experience of the interview, Applicants suppose that the Examiner means that the sequences of the IF, SakK, etc. genes are not expressly stated in the application. As to IF, the amino acid sequence of this peptide is given in Figure 2 and in the Sequence Listing. A nucleotide sequence encoding this

peptide is easily envisioned by the skilled artisan. As to SakK and SakR (and also IF), the nucleotide sequences of these genes from *L. sake* are provided by the GENBANK entry noted at page 3, line 26, now incorporated in the Sequence Listing. The nucleotide sequences of the *PlnA*, *PlnB*, *PlnC* and *PlnD* genes of *L. plantarum* C11 are known in the art, as explained previously (see, Diep et al. (1994)), and so need not be provided in the present specification. *Spectra-Physics, Inc. v. Coherent, Inc.* 3 USPQ2d 1737, 1743 (Fed. Cir. 1987). Thus, the molecules encompassed by these designations are indeed well-defined.

Applicants are not certain how it is that the phrase a gene "...of a lactic acid bacterium" (now "of a *Lactobacillus* species") would somehow encompass a gene not of a lactic acid bacterium. The phrase seems clear enough on its face. The Examiner seems to be extending "functional analogs" of IF, SakK and SakR, which can be the corresponding *Pln* genes from *L. plantarum*, as well as corresponding genes from both *Lactobacilli* and other genera, as somehow meaning that the *Pln* genes can be from a non-lactic acid bacterium. This is not a reasonable interpretation and is not intended by Applicants. As noted above, the *Pln* gene cluster is a well-defined nucleotide sequence and the products are well-defined.

Claims 120-123 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite for alleged failure to recite an

essential element. The Examiner asserts that the missing element is, "at least the SakR gene expression product." This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Applicants submit that the claims are definite as written within the requirements of 35 U.S.C. § 112, second paragraph. The alleged missing element is not "essential" as the Examiner asserts. Claims 120-123 are directed to kits. Claim 120 recites a vector and a peptide. Claim 122 recites two vectors. One of ordinary skill in the art would understand that a desired gene to be expressed would be cloned into the vector and then that expression vector would be transformed into a host cell of some kind. From reading of the specification, it is clear that use of host cell that is a *Lactobacillus* cell would provide the SakK and SakR gene products that are deemed essential by the Examiner. Culture of the transformed host in the presence of the peptide element recited in claim 120, or of the host cell co-transformed with both vectors of claim 122, would result in expression of the desired gene.

Furthermore, claims 121 and 123 recite that the kit further comprises a *Lactobacillus* host cell. Thus, the SakK and SakR gene expression products, or gene products that can serve their function, are implicit in these claims. Therefore, at least these claims should be found free of the instant rejection.

For all of the above reasons, Applicants respectfully submit that all of the present claims define patentable subject matter such that this application should be placed into condition for allowance. If there are minor issues precluding allowance of the application that can be addressed by a telephone discussion, the Examiner may contact the undersigned at the telephone number below, to discuss such matters.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) have petitioned for a two (2) month extension of time for filing a reply in connection with the present application in an accompanying Notice of Appeal, and the required fee of $205.00 is paid therewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and further replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. 1.16 or under 37 C.F.R. 1.17; particularly, extension of time fees.

Respectfully yours,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

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Attachments: Substitute Sequence Listing and diskette
Exhibits A-F

DRN/crt/rem

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The specification is amended at ph beginning at page 3, line 24:

--It is further published a paper by Tizacheck, P.S., Vogel, R.F. and Hammes, W.P., "Cloning and sequencing of SAKP encoding sakacin-P, the bacteriocin produced by Lactobacillus sake LTH673", Microbiology-UK (1994) V140, FEB (FEB), 361-67, and there is an entry in the EMBL/GenBank/DDBJ database (accession number Z48542) (SEQ ID NOS: 13-19) by Huehne, K., Holck, A., Axelsson L. and Kroeckel, L. (1995). These two publications describe the nucleotide sequences of pieces of DNA from, respectively, *Lactobacillus sake* LTH673, and *Lactobacillus sake* Lb674, that are, within the experimental error, identical and that encode genes involved in the production of bacteriocin, called sakacin P. These nucleotide sequences do also contain the promoter sequences depicted in Figure 4 (the upper four sequnces). However, these publications do only describe sequences; they do not describe: 1) the --

IN THE CLAIMS:

Claims 69, 71 77, 84, 86, 87, 89, 91, 93, 96, 98, 102, 124

and 125 have been canceled.

The claims have been amended as follows:

70. (Twice Amended) A gene expression system comprising:

(a) an IF gene of a *Lactobacillus* species;

(b) a SakK gene of a *Lactobacillus* species;

(c) a SakR gene of a *Lactobacillus* species;

(d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the expression product of the IF gene activates the expression product of the SakK gene, and

the activated expression product of the SakK gene activates the expression product of the SakR gene, and

the activated expression product of the SakR gene induces the first inducible promoter of the gene of interest,

thereby causing expression of the gene of interest;

wherein said the expression product of said IF gene is not a lantibiotic; and

wherein the IF gene is expressed from a promoter different from the promoter from which the SakK gene and/or the SakR gene are expressed; and

wherein the first inducible promoter comprises two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said

repeated sequence is located 30 to 38 nucleotides upstream from a -10 region of a bacterial gene, and wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, and residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.

73. (Amended) The gene expression system of claim 70 [69], wherein the SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof,] are operably linked to a constitutive promoter.

74. The gene expression system of claim 73, wherein the first inducible promoter and gene of interest are on a first vector and the SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof] are operably linked to a constitutive promoter located on a second vector separate from the first vector.

75. (Amended) The gene expression system of claim 70 [69], wherein the SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof,] are operably linked to a second inducible promoter.

76. (Amended) The gene expression system of claim 75, wherein the first inducible promoter and gene of interest are on a first vector and the SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof,] are operably linked to a second inducible promoter located on a second vector.

78. (Amended) A host cell comprising the gene expression system of claim [claims 69 or] 70.

90. (Amended) A kit for gene expression comprising:

a) at least one vector comprising (i) a promoter that can be induced by the expression product of a SakR gene of a [lactic acid bacterium] *Lactobacillus* species, wherein the SakR expression product is activated by the expression product of a SakK gene of a [lactic acid bacterium] *Lactobacillus* species, wherein the SakK expression product is activated by the expression product of an IF gene of a [lactic acid bacterium] *Lactobacillus* species, wherein the promoter comprises two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides upstream from a -10 region of a bacterial gene, and wherein said repeated nucleotide sequences are selected from the group

consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, and residues 7-8, 10-14 and 31-38 of SEQ ID NO:10; and (ii) a cloning site; and

b) a host strain having a chromosome comprising a SakK gene of a [lactic acid bacterium] *Lactobacillus* species and a SakR gene of a [lactic acid bacterium] *Lactobacillus* species.

92. (Amended) The kit of claim 90, further comprising c) a peptide comprising the amino acid sequence of residues 19-37 of SEQ ID NO:3[, or an analog thereof that can activate the expression product of a SakK gene or a functional analog of a SakK gene].

94. (Amended) A gene expression system comprising:

(a) an IF peptide[, or a functional analogue thereof];

(b) a SakK gene[, or a functional analogue thereof];

(c) a SakR gene[, or a functional analogue thereof];

(d) a cloned polynucleotide of interest linked to a first inducible promoter[,];

wherein in said gene expression system, the IF peptide, or functional analogue thereof activates the expression product of the SakK gene, or functional analogue thereof, and

the activated expression product of the SakK gene, or

functional analogue thereof, activates the expression product of the SakR gene, or functional analogue thereof, and

the activated expression product of the SakR gene, or functional analogue thereof, induces the first promoter of the gene of interest;

thereby causing expression of the gene of interest;

wherein the first inducible promoter is a promoter different from the promoter from which the SakK gene or functional analogue thereof and/or the SakR gene or functional analogue thereof are expressed.

95. (Amended) A gene expression system comprising:

(a) an IF peptide of a *Lactobacillus* species;

(b) a SakK gene of a *Lactobacillus* species;

(c) a SakR gene of a *Lactobacillus* species;

(d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the IF peptide activates the expression product of the SakK gene, and

the activated expression product of the SakK gene activates the expression product of the SakR gene, and

the activated expression product of the SakR gene induces the first promoter of the gene of interest,

thereby causing expression of the gene of interest;

wherein the first inducible promoter is a promoter different from the promoter from which the SakK gene [or functional analogue thereof] and/or the SakR gene [or functional analogue thereof] are expressed.

99. (Amended) The gene expression system of claim 95, wherein the first inducible promoter and gene of interest are on a first vector and the SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof] are operably linked to a constitutive promoter located on a second vector separate from the first vector.

100. (Amended) The gene expression system of claim 95 [94], wherein the SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof,] are operably linked to a second inducible promoter.

101. (Amended) The gene expression system of claim 95 [94], wherein the first inducible promoter and gene of interest are on a first vector and SakK gene[, or functional analog thereof,] and the SakR gene[, or functional analog thereof,] are operably linked to a second inducible promoter located on a second vector.

103. (Amended) A host cell comprising parts b), c) and d)

of the gene expression system of claim [claims 94 or] 95.

106. (Amended) A method for producing a polypeptide or protein of interest comprising culturing a host cell comprising the gene expression system of claim 103 in a medium, adding an IF peptide[, or functional analog thereof,] to the culture, thereby inducing expression of the gene of interest to produce the polypeptide or protein of interest; and

purifying the protein of interest from the culture.

107. (Twice Amended) An isolated nucleic acid comprising:

two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides upstream from a

-10 region of a bacterial gene,

wherein transcription of a coding nucleic acid sequence operatively linked to said isolated nucleic acid is activated by an expression product of a SakR gene of a *Lactobacillus* species [or functional analog thereof] that has been activated by an expression product of a SakK gene of *Lactobacillus* species [or functional analog thereof,] wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID

NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.

112. (Amended) A gene expression system comprising the vector of claim 109 and further comprising a polynucleotide encoding an IF peptide of a *Lactobacillus* species, a polynucleotide encoding a SakK protein of a *Lactobacillus* species and a polynucleotide [polynucletoide] encoding a SakR protein of a *Lactobacillus* species.

Claims 126-135 have been added.